EFFECT OF LONG ULTRAVIOLET AND SHORT VISIBLE RADIATION (3500 TO 4900Å) ON ESCHERICHIA COLI

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INTRODUCTION

The lethal effect of long ultraviolet (> 3500Å) and short visible (<4900Å) radiation on bacteria has been a controversial question for many years. Toxic effects of parts of this region of the spectrum have been reported by Ward (1894), Bayne-Jones and Van der Lingen (1923), and Coblentz and Fulton (1924). Negative results have been reported by Gates (1929), Ehrismann and Noethling (1932), and Bachem and Dushkin (1935). Additional literature is reviewed in a paper by Duggar (1936).

Carefully controlled experiments have made it possible to establish the conditions for the lethal action of the long ultraviolet and near-visible radiation. A study of certain sublethal effects gave an opportunity to distinguish the mechanism of the radiation action of wave lengths shorter than 3000Å and longer than 3500Å.

EXPERIMENTAL TECHNIQUE

The bacteria (*Escherichia coli*) were grown on nutrient agar (Difco) slants (20 hours at 37°C.), washed off with physiological salt solution,[1] shaken, filtered through absorbent cotton and centrifuged; and the sedimented organisms were resuspended in salt solution. They were then irradiated in phosphate buffer (pH 6.8), in a concentration of 200,000 to 1,000,000 organisms per ml. Exposures were made in Pyrex culture tubes, which were rotated by a simple mechanism. During the time of irradiation, both the exposed and the control tubes were kept in a constant-temperature bath.

Light sources used were either a General Electric H-6 lamp in a glass jacket or a water-cooled medium-pressure quartz capillary mercury vapor lamp of the Daniels-Heidt type (using 1 to 4 amp. at 150 to 240 volts). The radiation was concentrated, by means of a 500 ml. round-bottom flask filled with a water solution of copper sulfate ($CuSO_4 \cdot 5H_2O$—2 grams per liter) and a condensing lens, into a constant-temperature water tank with a glass window. The radiation also had to pass through two Corning Glass filters (# 738, 4 mm. and 585, 2 mm.) before entering the tank. (For details see figure 1.)

Spectrograms[2] of the incident radiation reaching the irradiated tube were taken with a quartz spectrograph, over a wide range of exposures, at the surface of the exposed culture tube. The photographs, even after very extended expo-

[1] Composition of physiologic salt solution: NaCl—3g, KCl—0.2 gr, $CaCl_2$—0.2 gr, 100 ml. distilled water.

[2] The spectrograms were taken by Dr. P. A. Cole of this Laboratory.

sures, showed no trace of radiation shorter than 3500Å and only very little above 4358Å in wavelength. Most of the energy was concentrated in the 3650Å set of lines; next in energy concentration came the 4358 group, and finally the 4046 set and some minor lines. The energy was determined by means of a thermopile connected with a high-sensitivity galvanometer, standardized against a National Bureau of Standards lamp. The exposure technique is very similar to that described by Hollaender, Brackett and Cole (1938).

Samples were removed from control and exposed tubes at certain intervals during exposure; these were diluted with physiological salt solution and plated out with nutrient agar, or incubated in nutrient broth or physiological salt solution and then plated out after certain time-intervals of incubation. At least three plates were poured for each dilution, and incubated for 48 hours at 37°C. All colonies were counted. Each of the counts given in the graphs and tables represent an average of at least three plates.

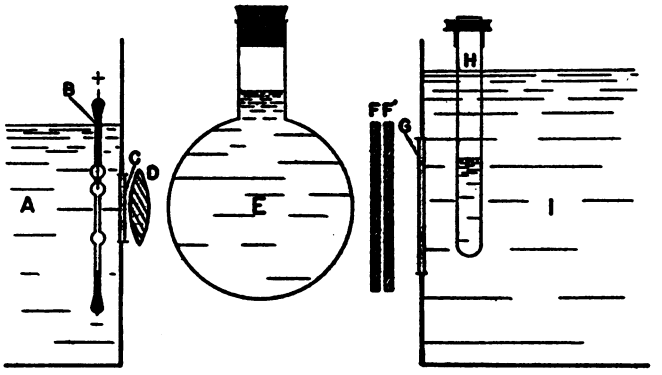

FIG. 1. EXPERIMENTAL ARRANGEMENT FOR IRRADIATION OF BACTERIA WITH λ 3500 TO 4900Å AT CONSTANT TEMPERATURE

A. Water bath for radiation source. B. Capillary mercury vapor lamp. C. Quartz window. D. Condensing lens. E. Round bottom flask filled with dilute solution of copper sulphate. F. Corning Glass filter #738, F' Corning Glass filter #585. G. Glass window. H. Exposed culture tube in rotating device. I. Constant temperature water bath.

RESULTS

A typical killing curve is given in figure 2. This figure also includes a graph showing the killing effect at wave length 2650Å. Attention is called to two distinct differences between these curves: (1) The incident energy necessary to produce 50 per cent killing at 3500–4900Å is 5×10^3 ergs/cm², while the energy necessary to bring about the same proportion of killing at 2650Å is about 10^3 ergs/cm². It requires, therefore, 10,000 to 100,000 times as much incident energy to kill at 3500–4900Å as at 2650Å. (2) The log survival ratio energy curve approaches a straight line for 2650Å and is decidedly of the threshold type for the long ultraviolet.

Temperature effect. The bactericidal action of wave lengths shorter than 3000Å has a temperature coefficient of about 1.1 (Gates, 1930). Radiation of 3500–4900Å has a temperature factor of about 1.7 to 2.2 (i.e. 10° rise in tem-

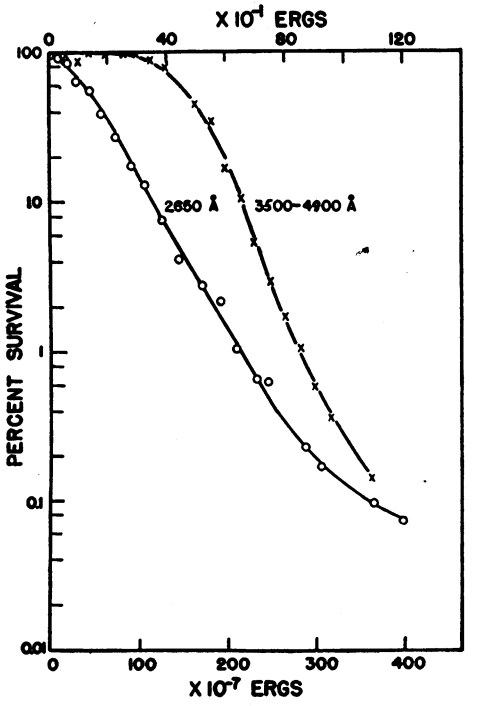

FIG. 2. SURVIVAL RATIO AGAINST ERGS/ORGANISM FOR E. COLI IN LIQUID SUSPENSION
Curve 3500 to 4900Å uses upper abscissa and 2650Å curve lower abscissa

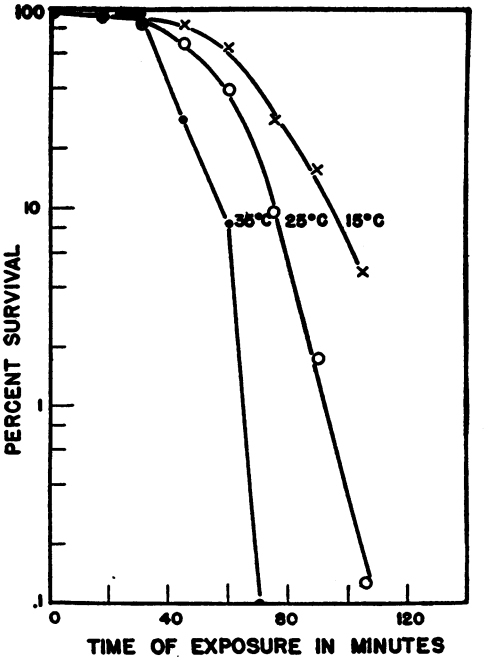

FIG. 3. CHANGE IN SURVIVAL RATIO WITH TIME FOR CONSTANT ENERGY (3500 TO 4900Å) AT THREE TEMPERATURES

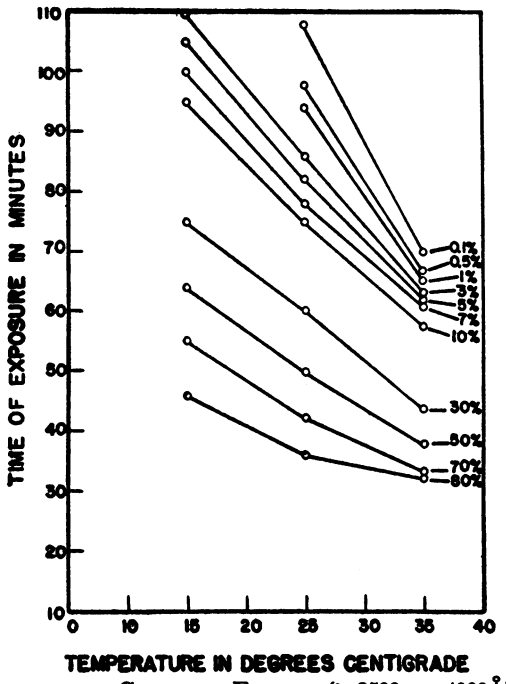

FIG. 4. TIME OF EXPOSURE AT CONSTANT ENERGY (λ 3500 TO 4900Å) PLOTTED AGAINST TEMPERATURE FOR A NUMBER OF SURVIVAL RATIOS, SHOWING THE INCREASING EFFECT OF TEMPERATURE ON LOW SURVIVAL RATIOS

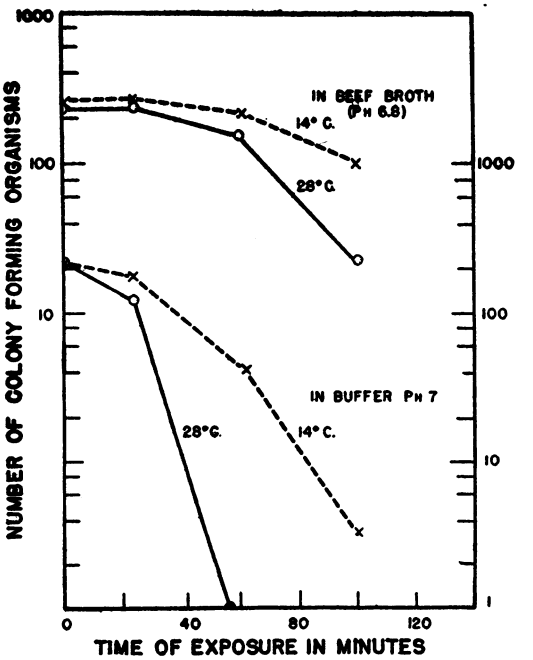

FIG. 5. CHANGE IN THE RELATIVE NUMBER OF COLONY-FORMING ORGANISMS WITH TIME OF EXPOSURE TO 3500 TO 4900Å IN EXPOSED (SOLID LINE) AND CONTROL (BROKEN LINE) CULTURE IN BEEF BROTH (LEFT SCALE) AND BUFFER SOLUTION (RIGHT SCALE)

perature reduces the energy necessary to kill by about one half). No effect of temperature is noticeable at the threshold part of the curve. However, as soon

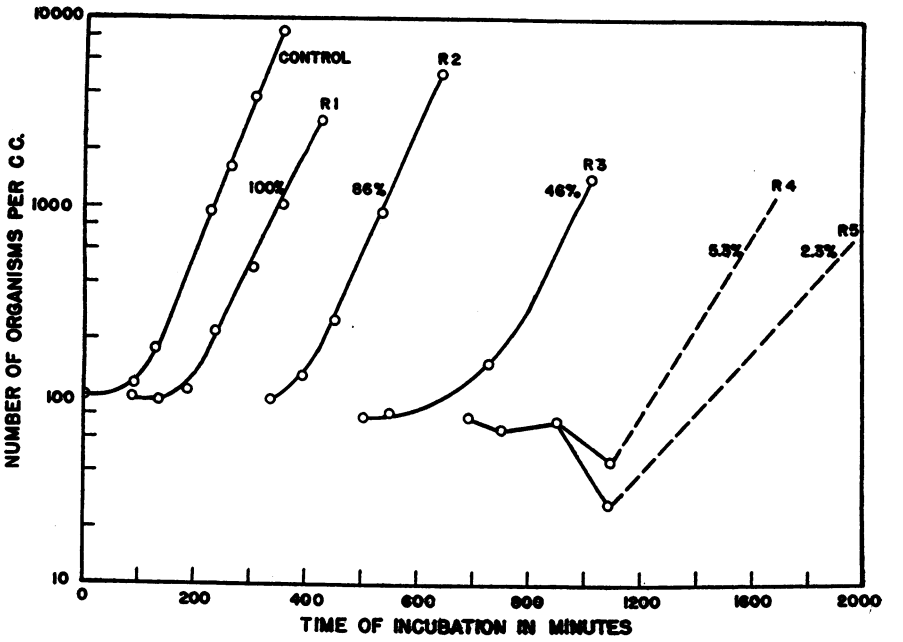

FIG. 6. CHANGE IN THE RELATIVE NUMBER OF COLONY-FORMING ORGANISMS PER CC. WITH TIME OF INCUBATION AFTER IRRADIATION, (WITH λ 3500 TO 4900Å) FOR FIVE SURVIVAL RATIOS AND CONTROL CULTURES

At low survival ratios the growth ratio was less reliable (broken lines). Details are given in the text.

TABLE 1

Effect of 3500 to 4900Å radiation on retarded-growth phase

CONTROL			RUN 2—SURVIVAL RATIO 86 PER CENT		
Time of incubation	No./ml.	Per cent	Time of incubation	No./ml.	Per cent
minutes			*minutes*		
0	379	100	0	325	100
82	444	117	90	322	102
127	749	176	150	339	104
217	3,560	940	210	289	92
262	5,900	1,556	270	306	94
307	14,500	3,820	330	309	95
367	31,800	8,400	390	420	129
			450	850	260
			525	3,020	930
			630	16,300	5,000

as the lethal action appears, i.e. after 40 minutes of exposure (see fig. 3) there is a very definite temperature effect which increases with the time of exposure. This is shown strikingly in figures 3 and 4.

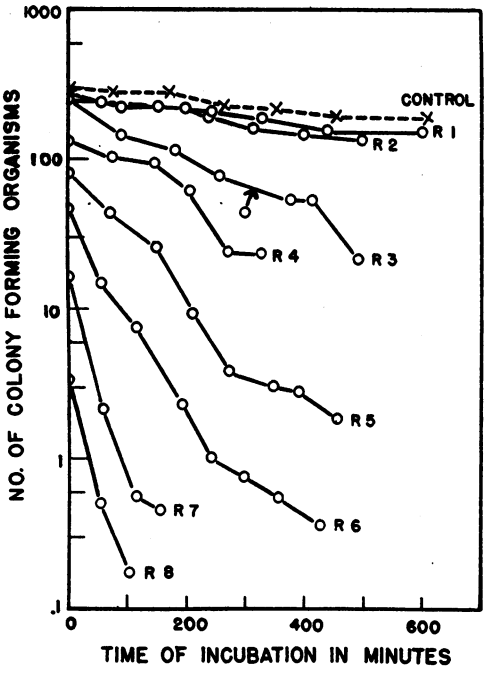

FIG. 7A. RELATIVE NUMBER OF COLONY-FORMING ORGANISMS AGAINST THE TIME OF INCUBATION IN PHYSIOLOGICAL SALT SOLUTION FOLLOWING IRRADIATION (3500 TO 4900A)

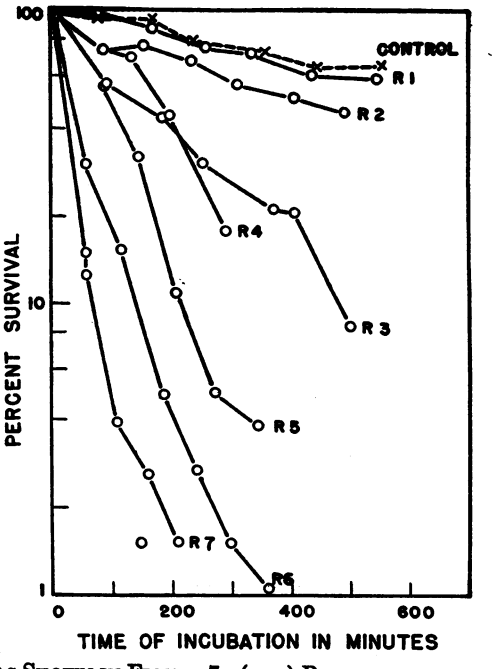

FIG. 7B. SAME DATA AS SHOWN IN FIGURE 7A (BUT) RECALCULATED ON A PERCENTAGE BASIS

The effect of irradiation in beef broth as compared with irradiation in buffer solution is shown in figure 5. Beef broth has a fairly high absorption coefficient in the near-ultraviolet region and thus protects the bacteria suspended in it to a considerable extent. The effect of salt solution is also discussed below.

Extension of "retarded growth phase." A study of the effect of ultraviolet radiation by wave lengths shorter than 3000Å on the extent of the "retarded growth phase" has provided interesting information concerning the function of the shorter ultraviolet (Hollaender and Duggar, 1938). Essentially the same technique for determining the growth rate was used with bacteria surviving irradiation with 3500 to 4900Å. The method consists in determining the number of colony-forming organisms by making plate counts immediately after irradiation and at certain intervals of incubation of the irradiated and control organisms in dilute beef broth. A typical set of growth curves is shown in figure 6. Data for the control and for Run 2 of this experiment are given in table 1. Each curve is marked with the survival ratio. The extension of the retarded growth phase begins before any organisms are killed by irradiation. With increasing energy, the delay in the initiation of cell division increases until a certain limit has been reached; after about 99 per cent of the organisms have been killed, no further extension of this phase appears to be possible.

Effect of salt solution. The "extended retarded growth phase" found after incubation in beef broth points to a "weakening" of bacteria that survive 3500 to 4900Å radiation. As a more definite check on this point, control and irradiated bacteria were suspended in physiological salt solution, and the number of colony-forming organisms was determined immediately after irradiation and at certain time-intervals of incubation. The results of a typical experiment are given in figures 7a and 7b. The control survived incubation in physiological salt solution very well for ten hours. The dying rate of bacteria after irradiation was substantially greater than of the control.

DISCUSSION

Outstanding findings on the lethal action of the region 3500–4900Å as compared with the region below 3000Å are given in table 2. Ultraviolet photographs of bacteria taken at wave lengths shorter than 3000Å show considerable detail, whereas photographs taken at the longer wave lengths show only general absorption (Wyckoff and Ter Louw, 1931), indicating that the compounds responsible for the effect of the longer wave lengths are probably diffused throughout the entire cell and are present in such small concentrations that they have no visible effect on the photographs taken. The extended threshold nature of the killing curve suggests the production of some toxic substance, or the destruction of some essential compound in the cell, the effect of which, up to a certain limit, does not permanently destroy the ability of the cell to divide and develop further. The presence of a high-temperature factor often indicates the existence of some secondary process. In general, photochemical reactions have low temperature coefficients. The region below 3000Å has a low coefficient, indicating that here the lethal effect results from a direct photochemical process. A dif-

fusion process, which might distribute a toxic compound formed by the radiation, would tend to have a high coefficient (see Belehradek, 1935).

The extensive prolongation of the so-called "lag phase" of the bacteria points definitely to the injurious effect of 3500–4900Å. It appears that this injurious effect is produced in a systematic manner, since a plot of the extension of the "retarded-growth phase," against energy gives a straight line. At the highest energy tested this straight-line relationship breaks down, indicating that the injury eventually becomes too great to permit recovery of the organisms. This injury to the bacteria expresses itself also in a lowered resistance of the organisms when suspended in physiological salt solution. It appears that the bacteria can obtain from nutrient beef broth (Difco) the material necessary for repairing the

TABLE 2

Effects of the 3500 to 4900Å and the 2180 to 2950Å region

	3500 TO 4900Å	2180 TO 2950Å
1. Shape of killing curve (log survival ratio/energy)	Threshold type	Approaching straight line
2. Energy (incident) for 50% survival ratio	Approximately 2×10^3 ergs/cm^2	5×10^2 to 10^3 ergs/cm^2
3. Temperature coefficient	1.7–2.2	1.1
4. Sublethal effects appear	Before any organisms are killed (in threshold part of killing curve)	After 60 to 90% of organisms are killed
5. Extension of retarded-growth phase for 10% survival ratio	Up to 1000%	50%
6. Toxicity of certain salt solutions can be recognized	At once after irradiation	In 600 minutes at 32°C.
7. Mutation production	No mutations	Mutations produced in fungi and Drosophila

cell, but that it is impossible for repair to take place without a supply of such material from outside the cell, as demonstrated when a suspension of physiological salt solution is used. Very little evidence can be found of the existence of a toxic substance producing the secondary effect observed after irradiation. The relative ease of recovery of irradiated organisms makes such an interpretation less probable, although the possibility cannot be eliminated. More support is available for the explanation that the radiation has destroyed some compound essential for the survival and multiplication of the bacterial cell. The extended retarded growth phase would then be the time necessary for the cell to replenish the essential material destroyed by the radiation. The dying rate in physiological salt solution would imply that the cell was not able to replace from other

cell constituents the compound destroyed by the radiation. In the case of relatively low energy values only a fraction of the "material" was effected by the radiation, and there was sufficient material left to keep the cell alive for a certain time in the non-nutrient salt solution.

A change in the morphology of the cell could be produced directly by the radiation; it also could be a secondary effect following the destruction of essential compounds necessary for the support of the cell. It is possible that in the destruction of an essential compound, toxic substances (see above discussion for limitations) are produced which interfere with the function of the cell, resulting finally in a change of the permeability of the cell wall or a change in some other physical structure of the cell. A relatively slight extension of the retarded-growth phase is also found after irradiation with wave lengths shorter than 3000Å. A possible explanation would be that the major effect of these wave lengths (< 3000Å) is through absorption in the nuclear material and that a residual effect is produced in the general protoplasm (Knaysi and Mudd, 1943). This latter effect might be similar to the one described for 3500 to 4900Å. (A detailed discussion of these points will be found in a forthcoming publication by Hollaender and Duggar.)

The bactericidal action of ultraviolet radiation of wave lengths shorter than 3000Å is most efficient at 2650Å, close to the wave lengths toward which nucleic acids are most highly absorbent. However, as far as has been tested, nucleic acid has no measurable absorption at wave lengths 3500 to 4900Å. Only relatively few biologically important compounds show definite absorption bands in this region of the spectrum. For instance, riboflavin has a set of bands in this region (Booker, 1939). It is also known that riboflavin becomes toxic after irradiation and is present in bacterial cells. We have added riboflavin to the suspension after irradiation of the bacteria, in the hope of shortening the radiation-produced extension of the "retarded-growth phase," but with no success. In all these experiments special care was taken to keep the bacteria dark after irradiation. Further tests on compounds, particularly respiratory enzymes which might be affected by the radiation, are now in progress.

Effects of irradiation with 3500–4900Å, similar to the ones described here for *E. coli*, have also been observed with other bacteria (*Eberthella typhosa*) also with yeast (Hollaender, Cole, and Brackett, 1938), fungi (Emmons and Hollaender), and nematode eggs (Jones and Hollaender, 1942). It is important to mention that no genetic effect of the range 3500–4900Å has been observed with fungi and *Drosophila* (Hollaender and Emmons, 1941); no systematic study on this point has been undertaken with bacteria.

The region of the spectrum from 3500 to 4900Å is of high intensity in sunlight. For instance, in Washington, D. C. on a clear day in July around noon-time, the intensity of this spectral region is approximately 4×10^4 ergs/cm²/sec. (Smithsonian Physical Tables, 1934). The intensity obtained in our laboratory with artificial sources is 3.5×10^6 ergs/cm²/min. According to these data, *E. coli* would be killed in a standard medium on a clear day at 25°C. in about 1 to 2 hours, if wave lengths below 3500Å were excluded. In tropical sunlight

the germicidal effect of natural radiation would be very high, especially if we consider that radiation below 3500Å and incidental infra-red radiation is added to the 3500–4900Å radiation. We will return in a further publication to the implications of the findings reported in this paper in relation to the hygienic and ecological influence of sunlight.

Since the completion of this manuscript, a report has been published on the effect of natural daylight and sunlight and artificial illumination on streptococci (Buchbinder, Salowey, and Phelps, 1941). Insofar as these studies can be compared with ours, they check quite well.

SUMMARY

1. A method for the irradiation of bacteria with measured quantities of 3500 to 4900Å is described.

2. The effect of the 3500 to 4900Å region on *Escherichia coli* is compared with the effect of radiation between 2000 and 3000Å with respect to the energy necessary to kill, shape of killing curve, temperature coefficient, extent of retarded-growth phase and survival after irradiation in physiological salt solution.

3. Possible mechanisms for the effect of radiation of 3500 to 4900Å are discussed.

REFERENCES

BACHEM, A., AND DUSHKIN, M. A. 1935 Study of bacterial sensitivity to ultraviolet radiation. Biol. Bull., **69**, 109–125.

BAYNE-JONES, S., AND VAN DER LINGEN, J. S. 1923 Bactericidal action of ultraviolet light. Bull. Johns Hopkins Hosp., **34**, 11–16.

BELEHRADEK, J. 1935 Temperature and living matter. Gebrüder Borntraeger, Berlin.

BOOHER, L. E. 1939 Chemical aspects of riboflavin in the vitamins. Am. Med. Assoc. Chicago.

BRACKETT, F. S., AND HOLLAENDER, ALEXANDER. June, 1939 Physical factors governing biological action of radiation. Phys. Rev., **55**, No. 11, 2nd series.

BUCHBINDER, L., SALOWEY, M., AND PHELPS, E. B. 1941 Studies on microorganisms in simulated room environments. III. The survival rates of streptococci in the presence of natural daylight and sunlight and artificial illumination. J. Bact., **42**, 353–366.

COBLENTZ, W. W., AND FULTON, H. R. 1924 A radiometric investigation of the germicidal action of ultraviolet radiation. U. S. Bur. Standards, Scientific Papers, No. 495, **19**, 641–680.

DUGGAR, B. M. 1936 Effects of radiation on bacteria in biological effects of radiation. McGraw-Hill Book Co., New York.

EHRISMANN, O. AND NOETHLING, W. 1932 Über die bactericide Wirkung monochromatischen Lichtes. Z. Hyg. Infektionskrankh., **113**, 597–628.

GATES, F. L. 1929 Study of the bactericidal action of ultraviolet light. J. Gen. Physiol., **13**, 231–248, 249–260; **14**, 31–42.

HOLLAENDER, A. 1940 The effects of long ultraviolet and near visible radiation on bacteria (abstract). Am. J. Botany, **27**, 165.

HOLLAENDER, A., AND CLAUS, W. D. 1936 The bactericidal effect of ultraviolet radiation on *Escherichia coli* in liquid suspensions. J. Gen. Physiol., **19**, 753–765.

HOLLAENDER, A., AND DUGGAR, B. M. 1938 Effects of sublethal doses of monochromatic ultraviolet radiation of growth properties of bacteria. J. Bact., **36**, 17–37.

HOLLAENDER, A., AND EMMONS, C. W. 1941 Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia Quant. Biol., **9**, 179–186.

HOLLAENDER, A., COLE, P. A., AND BRACKETT, F. S. 1939 Absorption and fluorescence spectra in relation to the photolethal action of methylcholanthrene on yeast. Am. J. Cancer, **37**, 265–272.

JONES, M. F., AND HOLLAENDER, A. 1942 Effect of long ultraviolet and near visible radiation on the eggs of the nematodes, *Enterobius vermicularis* and *Ascaris lumbricoides*. J. Parasitol., Suppl., **28**, 17–18.

KNAYSI, A., AND MUDD, S. 1942 The internal structure of certain bacteria as revealed by the electron microscope. A contribution to the study of the bacterial nucleus. J. Bact., **45**, 349–359.

SMITHSONIAN PHYSICAL TABLES. 1934 Smithsonian Institution, Wash., D. C.

WARD, H. M. 1893. The action of light on bacteria. Proc. Roy. Soc., (London) **52**, 393–400; **53**, 23–44.

WYCKOFF, R. W. G., AND TER LOUW, A. L. 1931 Some ultraviolet photomicrographs of *B. subtilis*. J. Exptl. Med., **54**, 449–451.